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REVIEW ARTICLE

Recent Progress on Colorimetric Sensors for Hg(II) Detection

M. S. Hakim[1,*], D. Hermayantiningsih[1], T. W. Manurung[1], M. Ariefin[1], and M. R. Bilad[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, 73111, Indonesia

[2]Faculty of Integrated Technologies, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE1410, Brunei Darussalam.

ABSTRACT – Mercury (Hg) contamination presents significant effects on human health and the environment, particularly caused by its bio-accumulative and neurotoxic properties. Effective detection of Hg(II) is essential for monitoring and mitigating its impact. Among various detection methods, colorimetric sensors have developed into a promising solution due to their affordability, simplicity, and real-time detection capabilities. This review summarizes recent advancements in colorimetric sensors for Hg(II) detection, focusing on innovative materials such as silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), paper-based substrates, agarose biopolymers, and carbon dots (CDs). AuNPs and AgNPs are highlighted for their unique optical properties and sensitivity, enabling visible detection through surface plasmon resonance changes. Paper-based sensors offer portability and low-cost solutions, while agarose serves as a stable biopolymer matrix for immobilizing detection agents. CDs, with their tunable fluorescence properties and green synthesis potential, provide a biocompatible option for hybrid sensing systems. Despite significant progress, challenges such as stability, selectivity in complex matrices, and scalability of green synthesis remain. Future research should focus on integrating these sensors into portable platforms and enhancing their performance for field applications. This review underscores the critical role of innovative materials and sustainable techniques in advancing mercury detection technologies.
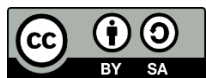
INTRODUCTION

Mercury, particularly its organic form, methylmercury, is one of the most toxic compounds to humans and the environment. It poses significant risks due to its unique toxicological properties. Methylmercury, a potent neurotoxin, bioaccumulates in aquatic food chains, magnifying its concentration at each trophic level and severely affecting humans through dietary exposure. The developing brain is particularly vulnerable, making prenatal exposure to methylmercury a leading cause of cognitive and neurological impairments in children. Even at lower than 5 µg/L, inorganic mercury has been reported to cause harmful effects [1], [2]. When inorganic mercury is transformed into methylmercury in marine environments, its toxicity amplifies, resulting in severe health issues such as allergic reactions and developmental delays in children exposed during pregnancy [3].

Mercury is released into the environment through both human activities and natural processes. Natural processes include geothermal activities, forest fires, weathering of mercury-containing rocks, and volcanic eruptions. However, human activities, particularly small-scale gold mining, are now the dominant sources of mercury emissions. The burning of mercury amalgam in these operations releases substantial amounts of mercury into the atmosphere, soil, and water, significantly disrupting the global mercury cycle. Indonesia, as one of the top three nations contributing to mercury emissions from gold mining, releases an estimated 340 tonnes of mercury annually, severely contaminating its environment [4].

Traditional mercury detection techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), cold vapour atomic-absorption spectrometry (CV-AAS), cold vapor-atomic fluorescence spectrometry (CV-AFS), and electrothermal atomic absorption spectrometry (ETAAS), are reliable for detecting mercury at trace levels [5]. However, these methods have disadvantages such as requiring a lot of chemicals, high analysis costs, and good operational skills. Most importantly, they cannot be used for in situ analysis. Colorimetric techniques visible to the human eye detect mercury pollution using modified gold nanoparticles. Among the several techniques developed for detecting mercury contamination, it is by far the most straightforward and rapid [6].

Colorimetric sensors offer the advantage of being visibly detectable to the naked eye. Their simplicity, affordability, and rapid detection capabilities make colorimetric sensor dyes and surface-modified metal nanoparticles particularly promising for future research. The colorimetric responses can be easily observed without complex equipment, either by using a ultraviolet-visible (UV-vis) spectrophotometer or the naked eye. Metal nanoparticles, particularly gold and silver, have shown significant interest due to their optical properties and high absorption coefficients that depend on

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size and shape [7]. They also meet the specifications of next-generation sensors, which include the potential for in-field detection and the advantages of being quick, easy, and affordable [8]. Additionally, colorimetric techniques are becoming increasingly popular for routine bioassays since they have superior selectivity and sensitivity to traditional techniques, often surpassing them [9]. In this review, we describe several potential materials for fabricating mercury sensors. In addition, this review also explains how results are synthesized, generated, and analyzed in mercury detection. Figure 1 shows a visual map with bibliometric analysis of keywords in published scientific articles. The analysis was carried out using the VOS Viewer 1.6.20 application on 81 keywords using the "Co-Accuracy" analysis type for publications published on Scopus. The larger circle created in the bibliometric analysis results via a visual map indicates more articles with related research.
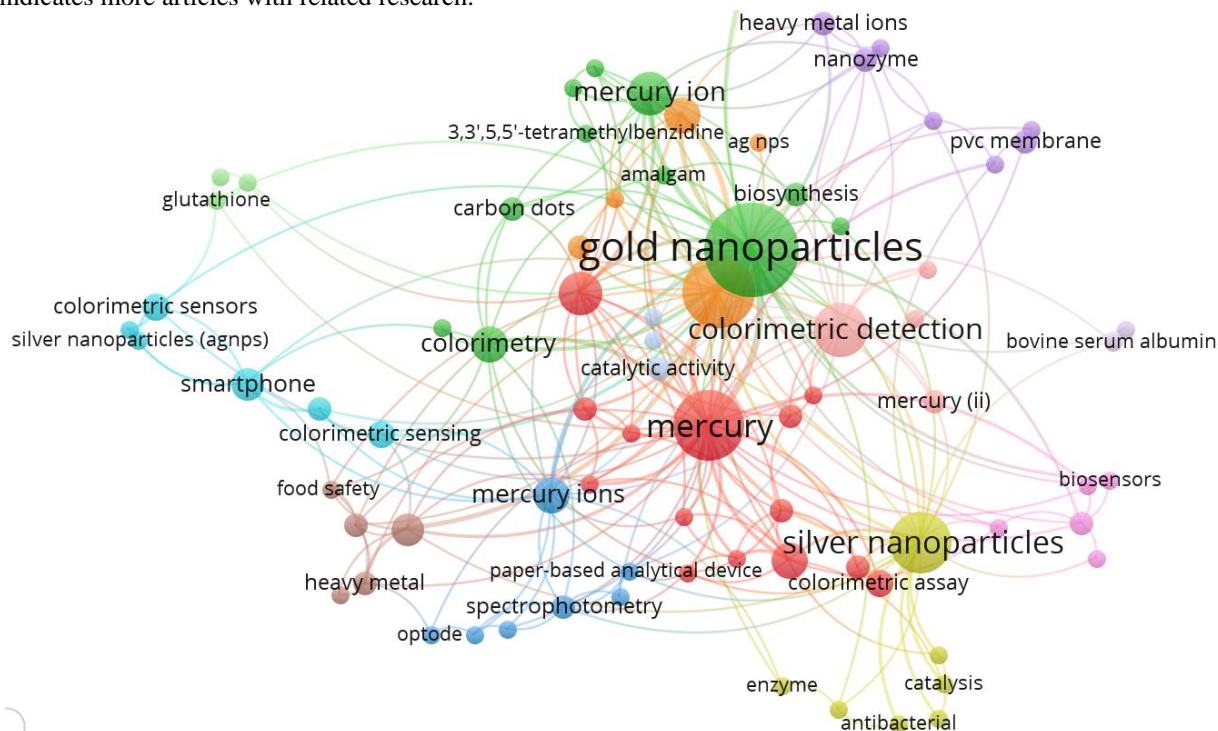

Figure 1. Visual map of keyword occurrences in scientific publications in Scopus
(Created with VOSviewers 1.6.20)

VARIOUS MATERIALS FOR Hg(II) SENSORS

This section covers the different materials used as matrices in the fabrication of mercury metal sensors. Every material has special qualities and benefits of its own. Advanced mercury sensing assays have been developed using gold nanoparticles (AuNPs), carbon dots (CDs), agarose, paper-based, and silver nanoparticles (AgNPs). Transduction techniques like colorimetric and fluorescence have been primarily used for signal readout. The percentage of publications on the use of materials as matrices in the creation of mercury sensors is shown in Figure 2.
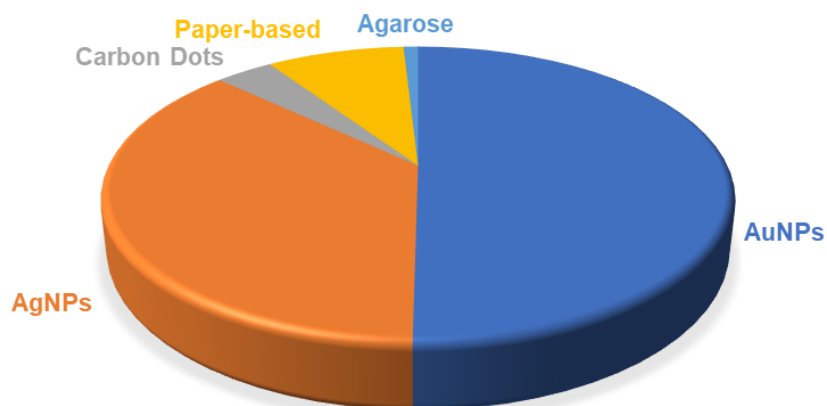

Figure 2. Percentage of publications during 2013–2023 in Scopus with the keyword "colorimetric sensor of mercury"

Gold Nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) are very popular due to their versatility in numerous applications such as imaging analysis, detection systems, and visual sensors. Given the severe hazards and detrimental impact on the environment and human health, the detection of mercury (II) or Hg(II) is typically carried out through visual means [10]. The current state of affairs necessitates the creation of quicker and simpler techniques for mercury contamination detection. Gold nanoparticles are essential to achieving this goal because of their superior adjustable optical characteristics.

Environmental techniques for creating AuNPs are growing in popularity as an alternative to chemical synthesis methods because of the importance of AuNPs in the sensing field. This has been accomplished by employing biological techniques to produce nanoparticles that can use plant extracts, fungi, or bacteria to reduce metal ions [11]. This technique has produced nanoparticles with remarkable control over morphology and size, despite being underutilized. It has been suggested that the aldehydes and ketones present in plant extracts can guide the formation of anisotropic AuNPs, enhancing their potential applications. However, despite the documentation of several biosynthesis pathways for producing AuNPs, the use of these biosynthesized nanoparticles in sensing remains limited [12].

The optical properties of AuNPs are greatly influenced by their aggregation state, shape, and size. It can be precisely controlled by choosing the stabilizing agent and appropriate synthesis method. Colloidal AuNPs are usually red or pink, but when they aggregate, they can turn purple-blue. This is the basis for colorimetric sensors, where the color changes and surface plasmon resonance (SPR) peak broadens as an effect of target analyte binding to the AuNPs surface and subsequent aggregation. SPR is a phenomenon in which a series of free electrons on the surface of a metal nanoparticle oscillate when exposed to light of a specific wavelength, resulting in a strong resonance and light absorption effect [13].

Because of their special optical and physical characteristics, AuNPs are utilized in biosensors and chemical applications. Due to their strong shape, high extinction coefficients, and optical characteristics, AuNPs are great options for creating colorimetric sensors, especially for metal ion sensors [14]. During the solution step, optical readouts are used to help get over the challenges posed by template-based approaches. Metallic nanoparticles are shown to be particularly useful in biological sensing, among the different colorimetric methods that are available. Mercury and other heavy metals can be effectively detected using metallic gold, particularly in its nano form [6]. Various detection methods, including aptamers and functionalized AuNPs nanosensors, have been reported. However, the success of AuNPs aggregation depends on the recognized ligand and the sample matrix ionic strength [15]. AuNPs alone do not change color; adding chemicals induces aggregation and color change.

Sener et al. [7] fabricated Hg(II) ions sensor for drinking water analysis that is easy to use, quick, and sensitive. It is useful and economical because it employs lysine as an AuNP aggregation promoter. The limit of detection (LOD) is less than the US Environmental Protection Agency limit, at about 2.9 nM. The assay has an advantage over techniques based on metal nanoparticles because it is very linear, quick, and affordable.

Zohora et al. [14] demonstrated how to create gold nanoparticles with tunable optical characteristics using an extract from the leaves of the *Cinnamomum tamala* plant. The amalgamation tendency of mercury with gold was utilized to detect Hg(II) using these nanoparticles. Additionally, the study discovered that during the synthesis of AuNPs, natural plant-based esters serve as capping agents.

By immobilizing cyanuric acid (CA)-conjugated gold nanoparticles (AuNPs) on filter paper, Saputri et al [16] created a Hg(II) sensor. This technique makes it possible to stabilize AuNPs against aggregation, which is essential for the sensor's operation. Using ImageJ software, which measured the red, green, and blue (RGB) values of the sensor's digital pictures, the intensity of the color shift was measured. This analysis provided a semi-quantitative assessment of the sensor's response (Figure 3).
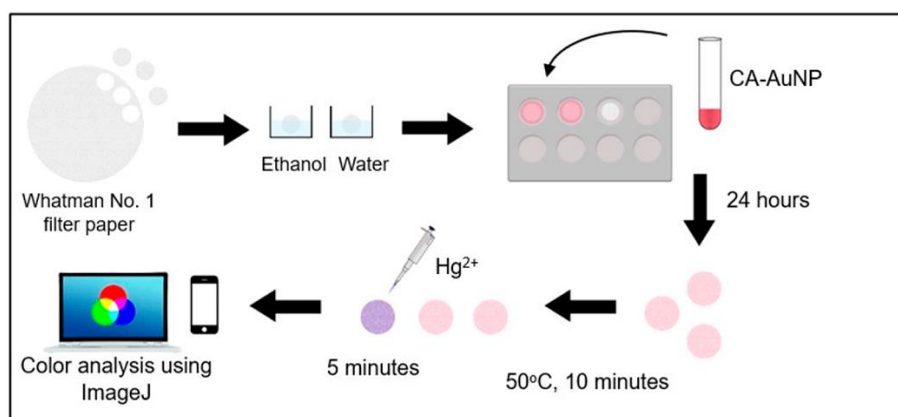

Figure 3. Schematic fabrication of Hg colorimetric sensor using cyanuric acid-conjugated gold nanoparticles [16] (Open Access CC-BY)

Silver Nanoparticles (AgNPs)

Silver (Ag) and other noble metal nanoparticles (NPs) have garnered increased attention lately because of their distinct physical and chemical properties as well as their numerous potential applications. AgNPs, or silver nanoparticles, are between one and one hundred nanometres in size. Their high surface area, small size, and ability to absorb near-infrared and visible light give them unique chemical characteristics. Because of their high surface-to-volume ratios and extremely small size, which cause physical and chemical changes, AgNPs may have additional antibacterial qualities not shown by ionic silver compared to their bulk counterparts [17]. The antibacterial activity of various metal nanoparticles, such as silver colloids, is directly related to their size; smaller silver nuclei exhibit greater antibacterial activity. Furthermore, the size distribution, shape, and structure of these nanoparticles, along with their physical and chemical environments, all impact their catalytic activity. Therefore, controlling the size distribution is crucial [18].

Two methods are commonly employed to produce silver nanoparticles: the "top-down" technique and the "bottom-up" approach. The "top-down" method involves mechanically grinding bulk metals and stabilizing them with colloidal protective agents. In contrast, the "bottom-up" approach relies on chemical and biological processes, where atoms self-assemble into new nuclei that eventually form nanoparticles. Common examples of "bottom-up" techniques include chemical reduction, electrochemical processes, and sono-decomposition. Meanwhile, "top-down" methods utilize various processes such as sputtering, grinding, milling, and thermal or laser ablation to reduce bulk materials into smaller fine particles [19].

The evaporation-condensation process is usually used in a tube furnace running at atmospheric pressure to produce nanoparticles using the "top to bottom" method. The primary material, which is placed in the middle of the furnace, is vaporized during this process to create a carrier gas inside a boat. This method's primary disadvantage is that the product's surface structure is flawed, and the nanoparticles' surface chemistry has a significant impact on their other physical properties [20].

AgNPs are now more widely used in colorimetric sensors due to their lower cost as compared to AuNPs. Additionally, AgNPs with a molar extinction coefficient 100 times higher may offer improved sensitivity. AgNP-based colorimetric sensors are currently receiving more attention [21]. AgNPs have been utilized as colorimetric sensors for detecting Hg(II) due to their strong attenuation coefficients and optical characteristics of the SPR absorption band [22].

The aggregation and surface modification of AgNPs causes colorimetric alterations. This can be accomplished by altering colloidal stabilization (non-crosslinking aggregation) or by NPs aggregating due to interparticle bond formation (cross-linking aggregation). The van der Waals attractive forces between NPs in the non-crosslinking system are what drive aggregation. Therefore, specific functional groups like hydroxyl (-OH), carboxyl (-COOH), and amine ($-NH_2$) on the surface of NPs are crucial for their capacity to aggregate. By modifying the NPs' surface chemistry and intermolecular ion strength, this method can be improved [22].

The morphology and size of AgNPs significantly influence their optical properties and the performance of the sensors. Smaller nanoparticles generally exhibit enhanced SPR responses due to their increased surface-to-volume ratio, facilitating interaction with analytes [23]. Chen et al. [24] reported a novel colorimetric sensor based on the morphological transformation of silver nanoprisms in the presence of Hg(II). This transition, triggered by the deprotection of capped molecules, resulted in a visible color change and a measurable blue shift in the SPR peak. The sensor demonstrated excellent linearity (10–500 nM), and the LOD was 3.3 nM, highlighting the critical role of nanoparticle morphology in sensor design.

To improve their sensitivity, unmodified silver nanoparticles must be modified with additional stabilizing agents because they usually show low sensitivity to Hg(II) in water. Their distinct optical characteristics, stability, water solubility, and capacity to combine with mercury (II) to form an amalgam make them generally an exploitable prospect [25]. AgNPs as a colorimetric Hg(II) sensor have recently been reviewed. The AgNP destabilizing agent used was 6-thioguanine. Furthermore, the presence of Hg(II) enhanced stability due to the strong interaction between 6-thioguanine and Hg(II). However, it was suggested that in the presence of Hg(II), lysine might be used to promote AuNP aggregation. Bhattacharjee and Chakraborty described an eco-friendly method for producing cystamine-stabilized AgNPs that uses a label-free colorimetric method for Hg(II) determination in water [26].

Mwakalesi et al. [27] used an aqueous extract from the root of *Synadenium glaucescens* (SYR) to create AgNPs. The process reduces silver ions and creates nanoparticles using secondary plant compounds found in the plant extract. The strong UV-vis spectrum at 420 nm, indicating the detection of a strong plasmon resonance, which denotes the formation of silver nanoparticles, verified the successful synthesis. It has been successfully demonstrated that the synthesized SYR-AgNPs can detect mercury ions in aqueous solutions. The procedure entailed introducing mercury ions into the silver nanoparticle solution and monitoring the resulting color shift. The color of SYR-AgNPs turned colorless upon the addition of mercury, and this change was directly correlated with the amount of mercury present (Figure 4).

The environmentally friendly synthesis of AgNPs under solar radiation was reported by Annadhasan et al [12]. With a detection limit of 16 nM, the synthesized AgNP solution shows high sensitivity to Hg and manganese (Mn). Using colorimetric techniques, this method can be applied to find heavy metal ions in aqueous media. Jarujamrus et al. [21] used the reduced tracer AgNP (unmodified AgNPs) to create a colorimetric sensor for the quick detection of Hg(II) ions. Transmission electron microscopy (TEM) revealed that the diameter of the synthesized AgNPs was 8.3 ± 1.4 nm. The absorbance of unmodified AgNPs shifted slightly to blue wavelengths and gradually decreased when the concentration of Hg(II) increased. This suggests that Hg(II) oxidized the AgNPs, causing the AgNPs in smaller particles to disintegrate and the reduction of Hg(II) to Hg(0), which is adsorbed on the AgNP surface and causes its reduction.
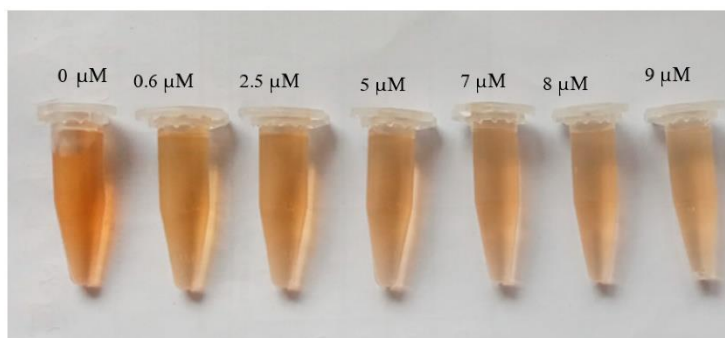
Figure 4. Color change of the AgNPs solutions in various concentrations of Hg [27]
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Paper-based

Paper-based sensors are among the innovations in the field of sensors that use paper as a substrate or base material [28]. This method is gaining increasing demand due to its advantages, such as portability, ease of use, low cost, and ability to be used in various areas, especially in detecting biochemical analytes in medicine and the environment [29]. Since the early 2000s, paper has been used as a sensing device. Within the previous decade, research on paper-based sensors has expanded to include new materials and more creative manufacturing processes. Due to its physical properties, it has become a versatile material that makes it widely used in many applications. According to several recent studies, paper-based material is suitable for use as a sensor base, including capillarity, flexibility, and the ability to facilitate various chemical reactions without complicated laboratory equipment [30].

Modifications of paper as a sensor material have been made. Specific modifications can increase functional groups on the paper surface, improve color uniformity for colorimetric sensors, and improve the stability of molecular immobilization. Several techniques used in paper surface modification are based on van der Waals forces, electrostatic bonding, physical interaction, and hydrogen bonding approaches. According to research by Thohir et al [31], the filter paper matrix's pores were filled with sol-gel that engaged with the supporting media's surface physically rather than chemically. The sol-gel matrix and filter paper physically interact, streamlining the manufacturing process and eliminating the need for binders or complicated chemical reactions. Evan et al. [32] reported that 3-aminopropyltriethoxysilane-modified silica nanoparticles were added to filter paper (Whatman grade no. 1). The colorimetric color gradient was affected by the adsorption of particular enzymes. Table 1 summarizes the paper-based material used as the sensor.

Applications of paper-based sensors in mercury detection often emphasize their field applicability. Devices using modified filter papers, such as those with chitosan-gold nanocomposites, have been effective for in situ mercury (II) or Hg(II) analysis. These sensors provide visual feedback in the form of color changes, which can be further analyzed using smartphone-based imaging tools, bridging the gap between laboratory and field environments. Additionally, innovations in reagent selection, as summarized in Table 1, highlight the range of materials employed to enhance the detection capabilities of paper-based sensors. These reagents include dithizone, rhodamine, and azine derivatives, which contribute to increased selectivity and lower detection limits for mercury ions. In conclusion, paper-based sensors offer a robust and low-cost solution for Hg(II) detection. Their adaptability to various functionalization techniques and compatibility with portable analytical tools position them as promising candidates for real-world applications. Future developments should focus on improving their long-term stability and extending their capabilities to detect mercury in more complex matrices.

Table 1. Various paper-based materials for the colorimetric sensor of Hg(II)

Paper Material	Reagent	LOD	Ref.
Filter paper	Cyanuric acid-conjugated gold nanoparticles	0.05 µM	[16]
Cellulose-based paper	1,4disubstituted azine	10 mg L^{-1}	[33]
Filter paper	Chitosan-gold nanocomposite	5.0×10^{-5} mM	[34]
Whatman filter No. 1	Dithizone	200 ppb	[35]
Filter paper	Dithizone	930 ppb	[36]
Filter paper	Rhodamine	27.2 ppb	[37]
Filter paper	Tetrahydrophenazine-based fluorophore	8×10^3 ppb	[38]
Filter paper	Rhodamine appended vinyl ether	1×10^4 ppb	[37]
Whatman No. 4 filter paper	HgI_4^{2-}	2×10^4 ppb	[39]

Agarose

Agarose is a biopolymer used extensively in heavy metal sensors and other sensor applications due to its advantageous qualities, such as chemical inertness, biocompatibility, and the capacity to form stable gels [40]. Many studies have examined the use of agarose in metal sensor development over the last ten years, particularly for the determination of hazardous metal ions like lead (II) or Pb(II) [41], mercury (II) or Hg(II) [42], and cadmium (II) or Cd(II) [43], which often pollutes the environment and is dangerous to human health. According to research by Alizadeh et al. [44], agarose has an excellent ability to be an immobilization matrix for detection substances (indicators) because it can form a pore network that can effectively absorb and retain metal ions. The use of agarose in metal sensors often involves combining it with supporting nanomaterials or chemical compounds to improve the selectivity and sensitivity of the sensor. A significant development is the combination of agarose with metal nanoparticles such as gold or silver [45]. Metal nanoparticles can also help amplify the optical or electrical signals produced when metal ions interact with agarose-based sensors, enabling the determination of metal ion concentration at very low levels, which is of great importance for water quality monitoring.

Over the last decade, there has been increasing use of the integration of agarose with technologies such as fluorescence and colorimetric sensors. For example, agarose fluorescent sensors have been applied to detect harmful metal ions through changes in fluorescence intensity when metal ions are bound to detection compounds immobilized in an agarose matrix [46]. Agarose-based colorimetric techniques also offer the advantage of easy visual detection, where color changes on the sensor indicate the presence of metal ions in the environment [47]. Although agarose has many advantages in metal sensing applications, challenges still exist regarding sensor stability and response time. Several recent studies have focused on structural modification of agarose to improve sensing performance, particularly in complex and dynamic environments. Chemical modifications to agarose, such as adding specific functional groups, can improve the sensor's ability to selectively detect certain metal ions without being influenced by other ions [48]. This is important in environmental sensing applications where different metal ions may be present in large quantities. Overall, the use of agarose in metal sensors continues to increase and has the potential to become an important tool in environmental monitoring and industrial applications.

Nshnsh et al. [49] used an agar-agar membrane and rhodamine B thiolactone (RBT) as a chromogenic reagent to create a naked-eye Hg sensor. When observed visually, the limit of detection (LOD) of the sensor was 0.4 µg L−1, and when examined digitally with image analysis software, it was 0.2 µg L-1. The sensors were successfully reused up to four times after being regenerated with potassium iodide KI 10% (w/v).

Carboxymethyl agarose-stabilized gold nanoparticles (CMA-AuNPs) were successfully synthesized by Chaudhary et al. [50] using an environmentally friendly method. The formation of the resultant nanoparticles was confirmed by their size range of 5 to 7 nm and their localized surface plasmon resonance (LSPR) absorption maximum at 523 nm. For Hg(II), the CMA-AuNPs demonstrated outstanding selectivity. Only Hg(II) significantly blue-shifted the LSPR band from 523 nm to 512 nm when interacting with 100 mg/L of various heavy metal ions.

Carbon Dots (CDs)

Carbon dots (CDs) are nanoparticles based on carbon atoms that can fluoresce and have unique capabilities such as low toxicity, biocompatibility, and tunable optical properties [51], [52], [53]. CDs can be synthesized from different precursors using different methods. Generally, CDs synthesis processes can be carried out using top-down and bottom-up methods [54], [55]. The size of CDs is less than 10 nanometers, and their structures vary based on the precursor and technique employed [56]. The optical properties of CDs, including photoluminescence and band gap, can be tuned through surface modification and the addition of heteroatoms as doping [57], [58]. Due to these various advantages, the use of CDs is very widespread, including drug delivery, bioimaging, catalysts, solar cell technology, and sensors [56], [59], [60].

Due to their ease of synthesis, fluorescence, and biocompatibility, CDs are quite popular nanoparticles used as sensors. Some CD system-based sensors have different mechanisms to act as metal sensors, such as reducing the fluorescence, increasing the fluorescence, or shifting the wavelength (Figure 5) [61]. As a metal sensor, CD offers several advantages, such as simplicity and high sensitivity, and allows it to be used as a portable tool [62], [63].
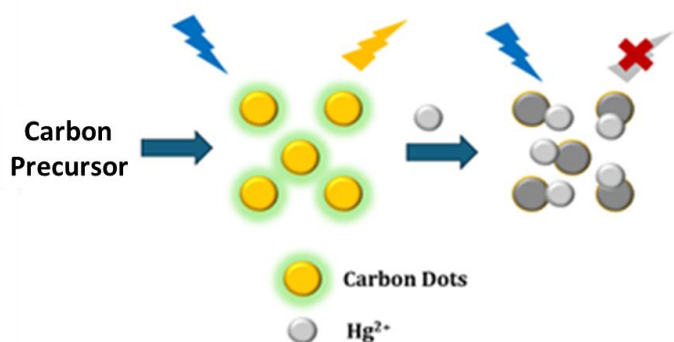

Figure 5. Turn off the CD-based sensor. Metal ions will quench the fluorescence of CDs

Several studies have reported the sensitivity and selectivity of CDs in detecting mercury (II) or Hg(II) ions in solution. Zhang et al. [64] developed switchable CDs-silver nanoparticles (CDs-AgNPs) composite materials for detecting Hg(II) ions. In this system, adding AgNPs to the CDs solution turns off the emission of Cd. However, when Hg(II) ions are added to the CDs-AgNPs mixture, the fluorescence in the solution returns; this method has a limit of detection (LOD) of 2.22×10^{-8} M. In addition, Liang et al. [65] also developed a CDs sensor made of sodium salicylate and ethylenediamine precursors. The addition of Hg(II) ions to the synthesized CDs solution eliminated the fluorescent properties of CDs, with the Hg test limit at a concentration of 0.05–10 µM. Table 2 summarizes various synthesis methods of CDs.

Table 2. Various synthesis methods of CDs

Precursors	Synthesis Methods	LOD (µM)	Ref.
Tamarindus indica	hydrothermal	6 nM	[66]
l-arginine	hydrothermal	0,188 µM	[61]
Polyethyleneimine, citric acid	hydrothermal	0,0033	[67]
Urea, EDTA	pyrolitic	0,0062	[68]
d-fructose and l-lysine	microwave	0,4	[69]
Citric acid, ethylenediamine, manganese borate	hydrothermal	0,0073	[70]
Orange juice, ethylenediamine	hydrothermal	-	[71]
folic acid and m-phenylenediamine (mPDA)	Hydrothermal	0,00215	[72]
folic acid, *p*-phenylenediamine (*p*PDA)	hydrothermal	0,00525	[72]
Sodium salicylate, ethylenediamine	hydrothermal	0,00440	[65]

CONCLUSION AND FUTURE PERSPECTIVE

Colorimetric sensors represent a promising approach to detecting mercury ions and offer the advantages of simplicity, cost-effectiveness, and rapid detection. Metal nanoparticles, particularly gold and silver, are effective in colorimetric tests due to their optical properties and responsiveness to Hg(II). Likewise, paper-based and agarose sensors show potential for environmental applications, while carbon dots offer a flexible platform for biocompatible sensors. However, challenges remain regarding stability, sensitivity, and selectivity, which are critical for real-world applications, especially in complex matrices such as natural waters. Addressing these limitations through innovative materials and synthesis techniques is essential to fully exploit the potential of colorimetric sensors in mercury monitoring.

Colorimetric sensors should focus on improving the specificity and sensitivity of mercury detection, ideally in the nanomolar range, to meet the needs of environmental and health monitoring. Integrating environmentally friendly synthesis methods and low-cost materials, such as plant-based stabilizers for nanoparticles, can improve accessibility and environmental sustainability. Additionally, the development of portable devices containing these colorimetric sensors, such as smartphone-based analyzers, could facilitate on-site testing. By further developing user-friendly, highly sensitive, and selective systems, colorimetric sensors could become an important tool for mitigating the risks associated with mercury contamination.

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